

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|-----------|--|
| (51) International Patent Classification ⁶ : C12Q 1/68, G01N 33/574 | A1 | (11) International Publication Number: WO 99/60162 (43) International Publication Date: 25 November 1999 (25.11.99) |
| (21) International Application Number: PCT/US99/10548 (22) International Filing Date: 12 May 1999 (12.05.99) (30) Priority Data: 60/086,265 21 May 1998 (21.05.98) US (63) Related by Continuation (CON) or Continuation-In-Part (CIP) to Earlier Application US 60/086,265 (CIP) Filed on 21 May 1998 (21.05.98) (71) Applicant (for all designated States except US): DIADEXUS LLC [US/US]; 3303 Octavius Drive, Santa Clara, CA 95054 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): ALI, Shujath [IN/US]; Apartment 357, 3475 Granada Avenue, Santa Clara, CA 95051 (US). SALCEDA, Susana [AR/US]; 4118 Cresendo Avenue, San Jose, CA 95136 (US). SUN, Yongming [CN/US]; Apartment 260, 869 S. Winchester Boulevard, San Jose, CA 95128 (US). CAFFERKEY, Robert [IE/US]; Apartment 4305, 651 Franklin Street, Mountain View, CA 94041 (US). | | (74) Agents: LICATA, Jane, Massey et al.; Law Offices of Jane Massey Licata, 66 E. Main Street, Marlton, NJ 08053 (US). (81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> |
| (54) Title: A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING PROSTATE CANCER (57) Abstract <p>The present invention provides a new method for detecting, diagnosing, monitoring, staging and prognosticating prostate cancer.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

- 1 -

**A NOVEL METHOD OF DIAGNOSING,
MONITORING, AND STAGING PROSTATE CANCER**

FIELD OF THE INVENTION

This invention relates, in part, to newly developed
5 assays for detecting, diagnosing, monitoring, staging, and
prognosticating cancers, particularly prostate cancer.

BACKGROUND OF THE INVENTION

Cancer of the prostate is the most prevalent
malignancy in adult males, excluding skin cancer, and is an
10 increasingly prevalent health problem in the United States.
In 1996, it was estimated that in the United States, 41,400
deaths would result from this disease, indicating that
prostate cancer is second only to lung cancer as the most
common cause of death in the same population. If diagnosed
15 and treated early, when the cancer is still confined to the
prostate, the chance of cure is significantly higher.

Treatment decisions for an individual are linked to
the stage of prostate cancer present in that individual. A
common classification of the spread of prostate cancer was
20 developed by the American Urological Association (AUA). The
AUA classification divides prostate tumors into four stages,
A to D. Stage A, microscopic cancer within prostate, is
further subdivided into stages A1 and A2. Sub-stage A1 is a
well-differentiated cancer confined to one site within the
25 prostate. Treatment is generally observation, radical
prostatectomy, or radiation. Sub-stage A2 is a moderately to
poorly differentiated cancer at multiple sites within the
prostate. Treatment is radical prostatectomy or radiation.
Stage B, palpable lump within the prostate, is further
30 subdivided into stages B1 and B2. In sub-stage B1, the cancer
forms a small nodule in one lobe of the prostate. In sub-
stage B2, the cancer forms large or multiple nodules, or
occurs in both lobes of the prostate. Treatment for both sub-
stages B1 and B2 is either radical prostatectomy or radiation.

- 2 -

Stage C is a large cancer mass involving most or all of the prostate and is further subdivided into two stages. In sub-stage C1, the cancer forms a continuous mass that may have extended beyond the prostate. In sub-stage C2, the cancer
5 forms a continuous mass that invades the surrounding tissue. Treatment for both these sub-stages is radiation with or without drugs. The fourth stage is metastatic cancer and is also subdivided into two stages. In sub-stage D1, the cancer appears in the lymph nodes of the pelvis. In sub-stage D2,
10 the cancer involves tissues beyond lymph nodes. Treatment for both these sub-stages is systemic drugs to address the cancer as well as pain.

However, current prostate cancer staging methods are limited. As many as 50% of prostate cancers initially staged
15 as A2, B, or C are actually stage D, metastatic. Discovery of metastasis is significant because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers. The five year survival rates for patients with localized and metastatic
20 prostate cancers are 93% and 29%, respectively.

Accordingly, there is a great need for increasingly sensitive methods for the staging of a cancer in a human to determine whether or not such cancer has metastasized and for monitoring the progress of a cancer in a human.

25 In the present invention, methods are provided for detecting, diagnosing, monitoring, staging and prognosticating cancers, particularly prostate cancer via seven (7) Prostate Specific Genes (PSG). The seven PSGs refer, among other things, to native proteins expressed by the genes comprising
30 the polynucleotide sequences of any of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. In the alternative, what is meant by the seven PSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7 or levels of the genes comprising

- 3 -

any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill
5 in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the
10 disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the
15 present invention to provide a method for diagnosing the presence of prostate cancer in a patient which comprises measuring levels of PSG in a sample of cells, tissue or bodily fluid from the patient and comparing the measured levels of PSG with levels of PSG in preferably the same cells, tissue,
20 or bodily fluid type of a control, wherein an increase in the measured PSG levels in the patient versus levels of PSG in the control is associated with prostate cancer.

Another object of the present invention is to provide a method of diagnosing metastatic prostate cancer in
25 a patient which comprises measuring PSG levels in a sample of cells, tissue, or bodily fluid from the patient and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured PSG levels in the patient versus
30 levels of PSG in the control is associated with a cancer which has metastasized.

Another object of the present invention is to provide a method of staging prostate cancer in a patient which comprises identifying a patient having prostate cancer,

- 4 -

measuring levels of PSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue or bodily fluid type of a control. An increase
5 in measured PSG levels in the patient versus PSG levels in the control can be associated with a cancer which is progressing while a decrease or equivalent level of PSG measured in the patient versus the control can be associated with a cancer which is regressing or in remission.

10 Another object of the present invention is to provide a method of monitoring prostate cancer in a patient for the onset of metastasis. The method comprises identifying a patient having prostate cancer that is not known to have metastasized, periodically measuring levels of PSG in a sample
15 of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured PSG levels versus control PSG levels is associated with a cancer which has
20 metastasized.

Yet another object of the present invention is to provide a method of monitoring the change in stage of prostate cancer in a patient which comprises identifying a patient having prostate cancer, periodically measuring levels of PSG
25 in a sample of cells, tissue, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a control wherein an increase in measured PSG levels versus the control PSG levels is associated with a cancer
30 which is progressing and a decrease in the measured PSG levels versus the control PSG levels is associated with a cancer which is regressing or in remission.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill
35 in the art from the following description. It should be

- 5 -

understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the
5 disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays
10 and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging, and prognosticating cancers by comparing levels of PSG measured in a patient with levels of PSG in a control. What is meant by "levels of PSG" as used herein, means levels of the native protein expressed by the
15 gene comprising the polynucleotide sequence of any of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. In the alternative, what is meant by "levels of PSG" as used herein, is levels of the native mRNA encoded by the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7 or levels of the
20 gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. Such levels are preferably measured in at least one of cells, tissues and/or bodily fluids, and includes determination of both normal and abnormal levels of PSGs. Thus, for instance, a diagnostic assay in
25 accordance with the invention for diagnosing overexpression of PSG protein compared to control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including prostate cancer. Any of the seven PSGs may be measured alone in the methods of the invention, all
30 together or in various combinations of the seven PSGs.

By "control" it is meant a human patient without cancer and/or non cancerous samples from the patient, also referred to herein as a normal human control; in the methods for diagnosing or monitoring for metastasis, control may also

- 6 -

include samples from a human patient that is determined by reliable methods to have prostate cancer which has not metastasized.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as PSG. Other cancer markers, in addition to PSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art. For example, simultaneous testing for increases in PSA as well as increases in PSG are also within the scope of the present invention and believed to provide a higher level of assurance that such cancer being tested is metastatic or the onset of metastasis has occurred.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of prostate cancer by analyzing for changes in levels of PSG in cells, tissues or bodily fluids compared with levels of PSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of PSG in the patient versus the normal human control is associated with the presence of prostate cancer. Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as PSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic prostate cancer in a patient having prostate cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having prostate cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a

- 7 -

variety of means known to those of skill in the art. For example, in the case of prostate cancer, patients are typically diagnosed with prostate cancer following traditional detection methods.

5 In the present invention, determining the presence of PSG in cells, tissues, or bodily fluid, is particularly useful for discriminating between prostate cancer which has not metastasized and prostate cancer which has metastasized.

Existing techniques have difficulty discriminating
10 between prostate cancer which has metastasized and prostate cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissue, or bodily fluid are PSGs, and
15 are compared with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just PSG in serum, this level is preferably compared with the level of PSG in serum of a normal human patient. An increase in the PSG in
20 the patient versus the normal human control is associated with prostate cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored
25 has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as PSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

30 **Staging**

The invention also provides a method of staging prostate cancer in a human patient.

The method comprises identifying a human patient having such cancer and analyzing a sample of cells, tissues,
35 or bodily fluid from such patient for PSG. Then, the method

- 8 -

compares PSG levels in such cells, tissues, or bodily fluid with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in PSG levels in the patient versus the normal
5 human control is associated with a cancer which is progressing and a decrease in the levels of PSG is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring prostate
10 cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for PSG; and comparing the PSG levels
15 in such cells, tissue, or bodily fluid with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in PSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

20 Further provided by this invention is a method of monitoring the change in stage of prostate cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissue, or bodily fluid from such patient for PSG;
25 comparing the PSG levels in such cells, tissue, or bodily fluid with levels of PSG in preferably the same patient.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the
30 particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression, such as PSG of the present invention, in a sample derived from a host are well-known to those of skill
35 in the art. Such assay methods include radioimmunoassays,

- 9 -

reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses and ELISA assays. Among these, ELISAs are frequently preferred to
5 diagnose a gene's expressed protein in biological fluids. An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to PSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to
10 PSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to PSG is
15 incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time PSG binds
20 to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to PSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to PSG.
25 Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to PSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to
30 the amount of PSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to PSG attached to a solid support and
35 labeled PSG and a sample derived from the host are passed over

- 10 -

the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of PSG in the sample.

Nucleic acid methods may be used to detect PSG mRNA as a marker for prostate cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the PSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the PSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest.

Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of

- 11 -

gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and
5 then using that material to generate a standard curve.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in
10 the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

EXAMPLES

15 The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or
20 circumscribe the scope of the disclosed invention.

EXAMPLE 1: PSGs

Searches were carried out and PSGs identified using the following Search Tools as part of the LIFESEQ® database available from Incyte Pharmaceuticals, Palo Alto, CA:

25 1. Library Comparison (compares one library to one other library) allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue.

30 2. Subsetting is similar to library comparison but allows the identification of clones expressed in a pool of libraries and absent or expressed at a lower level in a second pool of libraries.

- 12 -

3. Transcript Imaging lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones can then be examined using Electronic Northern to determine the tissue sources of their component
5 ESTs.

4. Protein Function: Incyte has identified subsets of ESTs with a potential protein function based on homologies to known proteins. Some examples in this database include Transcription Factors and Proteases. Some leads were
10 identified by searching in this database for clones whose component ESTs showed disease specificity.

Electronic subtractions, transcript imaging and protein function searches were used to identify clones, whose component ESTs were exclusively or more frequently found in
15 libraries from specific tumors. Individual candidate clones were examined in detail by checking where each EST originated.

Table 1:

| SEQ ID NO: | Clone ID # | Gene ID # | Protein Function (Transcription Factors) |
|---------------|------------|--------------|---|
| | | | |
| 1 | 1550426 | 244673 | |
| 2 | 1255804 | 14878 | Subsetting |
| 3 | 1808432 | 255819 | Subsetting |
| 4 | 3930803 | none | Subsetting |
| 5 | 645804 | 235032 | Subsetting |
| 6 | 1862352 | 221558 | Subsetting |
| 7 | 1450626 | 236019 | Subsetting |

**EXAMPLE 2: Measurement of SEQ ID NO:1; Clone ID # 1550426;
Gene ID #244673 (pro101)**

30 The example is carried out using standard techniques, which are well known and routine to those of skill in the art,

- 13 -

except where otherwise described in detail. Routine molecular biology techniques of the following example are carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Relative Quantitation of Gene Expression

Real-time quantitative PCR with fluorescent Taqman probes is a quantitative detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample are used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" is obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

To evaluate the tissue distribution, and the level of pro101 (SEQ ID NO:1) in normal and tumor tissue, total RNA was extracted from tumor and matched normal adjacent tissues and from unmatched tumor and normal tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction carried out using primers and Taqman probe specific to pro101 (SEQ ID NO:1). The results

- 14 -

were obtained using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of pro101 (SEQ ID NO:1) compared to the calibrator.

The absolute numbers are depicted in the following Table 2 as relative levels of expression in 12 normal tissues of pro101 (SEQ ID NO:1) compared to kidney (calibrator). These RNA samples were generated by pooling samples from a particular tissue from different individuals.

Table 2: Relative levels of pro101 Expression in Pooled Samples

| Tissue | NORMAL |
|---------------|---------------|
| Brain | 1.2 |
| Heart | 2 |
| Kidney | 1 |
| Liver | 7.2 |
| Lung | 48.2 |
| Mammary | 2.5 |
| Prostate | 1418.4 |
| Spleen | 1.6 |
| Small | 1.9 |
| Testis | 57.3 |
| Thymus | 1.3 |
| Uterus | 7.6 |

The relative levels of expression in Table 2 show that for the PSG pro101 (SEQ ID NO:1) mRNA expression is more than 20 fold higher in the pool of normal prostate compared with the other 11 normal tissue pools analyzed. These results demonstrate that mRNA expression of the PSG is highly specific for prostate.

The tissues shown in Table 2 correspond to pools of samples from different individuals. The tissues shown in the following Table 3 were obtained from individuals and are not pooled. Hence the values for mRNA expression levels shown in Table 2 cannot be directly compared to the values shown in Table 3.

- 15 -

The absolute numbers in Table 3 are relative levels of expression of prol01 (SEQ ID NO:1) compared to kidney (calibrator), in 60 pairs of matching samples. Each matching pair contains the cancer sample for a particular tissue and the normal adjacent sample for that same tissue from the same individual. The results from 3 unmatched ovary tumor, 3 unmatched normal ovary, 1 unmatched mammary tumor and 1 unmatched normal mammary gland are also shown.

Table 3: Relative Levels of prol01 Expression in Individual Samples

10

15

20

25

30

35

40

45

| TISSUE | CANCER | MATCHING | UNMATCHED |
|-------------|---------|----------|-----------|
| Prostate 1 | 103.9 | 0 | |
| Prostate 2 | 2219 | 84.2 | |
| Prostate 3 | 5048.2 | 3623.6 | |
| Prostate 4 | 11052.3 | 2029.4 | |
| Prostate 5 | 229.1 | 41.1 | |
| Prostate 6 | 57.9 | 25.3 | |
| Prostate 7 | 58.5 | 57.069 | |
| Prostate 8 | 1074.6 | 610.8 | |
| Prostate 9 | 32.7 | 79.3 | |
| Prostate 10 | 15.8 | 2.09 | |
| Prostate 11 | 436.4 | 438 | |
| Prostate 12 | 49.5 | 59.3 | |
| Prostate 13 | 128 | 56 | |
| Bladder 1 | 0 | 0 | |
| Bladder 2 | 0 | 0 | |
| Bladder 3 | 0.7 | 0 | |
| Colon 1 | 0 | 0 | |
| Colon 2 | 0 | 0 | |
| Colon 3 | 0 | 0 | |
| Colon 4 | 3.3 | 1.9 | |
| Colon 5 | 0.1 | 0.8 | |
| Colon 6 | 0 | 0 | |
| Lung 1 | 0 | 0 | |
| Lung 2 | 0.5 | 1.6 | |
| Lung 3 | 1.4 | 2.1 | |
| Lung 4 | 0 | 0 | |
| Lung 5 | 0 | 0 | |
| Kidney 1 | 0 | 0 | |
| Kidney 2 | 0 | 0 | |
| Kidney 3 | 0 | 0 | |
| Kidney 4 | 0 | 0 | |
| Liver 1 | 1.5 | 5.7 | |
| Liver 2 | 26.9 | 7.9 | |
| Liver 3 | 0 | 0 | |

- 16 -

| | | | | |
|----|------------|-----|-----|------|
| | Pancreas 1 | 0.9 | 0.9 | |
| | Pancreas 2 | 3 | 0 | |
| | Pancreas 3 | 0 | 0 | |
| | Pancreas 4 | 0 | 0 | |
| 5 | Pancreas 5 | 0 | 0 | |
| | Stomach 1 | 0 | 0 | |
| | Stomach 2 | 0 | 0 | |
| | Stomach 3 | 0 | 0 | |
| | Stomach 4 | 0 | 0 | |
| 10 | Stomach 5 | 0 | 0 | |
| | Sm Int 1 | 0 | 0 | |
| | Sm Int 2 | 0 | 0 | |
| | Testis 1 | 0 | 0 | |
| | Mammary 1 | 4 | 0 | |
| 15 | Mammary 2 | 5.6 | 0 | |
| | Mammary 3 | 0.5 | 0 | |
| | Mammary 4 | 0.4 | 0 | |
| | Mammary 5 | 0.5 | | |
| | Mammary 6 | | | 0 |
| 20 | Endo 1 | 1.6 | 7.6 | |
| | Endo 2 | 0 | 0 | |
| | Endo 3 | 0 | 0 | |
| | Endo 4 | 0.3 | 0.2 | |
| | Endo 5 | 5.8 | 5 | |
| 25 | Uterus 1 | 0 | 0 | |
| | Uterus 2 | 0 | 0 | |
| | Uterus 3 | 0 | 0 | |
| | Uterus 4 | 2.2 | 2.6 | |
| | Ovary 1 | 1.4 | | |
| 30 | Ovary 2 | | | 11.6 |
| | Ovary 3 | 1.5 | | |
| | Ovary 4 | | | 22.9 |
| | Ovary 5 | 0 | | |
| | Ovary 6 | | | 1.8 |

35 Among 128 samples in Table 3 representing 14 different tissues, the higher levels of expression are consistently in prostate tissues. These results confirm the tissue specificity results obtained with normal samples shown in Table 2. Table 2 and Table 3 represent a combined total of

40 140 samples in 18 human tissue types. Sixty-eight samples representing 13 different tissue types excluding prostate had no detected pro101 mRNA (Table 3). In 4 tissues (stomach small intestine kidney and testis) no pro101 (SEQ ID NO:1) mRNA was detected for any sample tested from individuals

45 (Table 3). Expression of this PSG was detected in testis in the pooled normal sample (Table 3). The median expression in

- 17 -

prostate cancer samples in Table 3 is 166.5 units. Excluding Ovary 4 (Normal), only 1 sample in Table 3, Liver 2 (Cancer), is greater than 10% of this value.

Comparisons of the level of mRNA expression in prostate tumor samples and the normal adjacent tissue from the same individuals are also shown in Table 3. The PSG prol01 (SEQ ID NO:1) is expressed at higher levels in 9 of 13 (69%) prostate cancer tissues (Prostate 1, 2, 3, 4, 5, 6, 8, 10 and 13) compared with the corresponding normal adjacent tissue. The level of expression of this PSG is lower in prostate tumor compared to normal adjacent tissue in two samples (Prostate 9 and 12). Equivalent levels of expression were detected in two matched samples (Prostate 7 and 11). Previous mRNA expression analysis for genes coding for the diagnostic markers PSA and PLA2 showed higher expression of the mRNA in 40% to 80% of the tumor samples compared to matching normal adjacent tissue. Higher expression in the tumor sample compared to the corresponding normal adjacent tissue is observed for Bladder 3, Colon 4, Liver 2, Pancreas 2, Endometrium 5 and. Mammary 1, 2 and 3. Higher expression in the normal adjacent samples is observed for Colon 5, Lung 2, Lung 3, Liver 1, Endometrium 1 and Uterus 4. However, the levels detected are in most cases comparable amongst the different tissues and low compared to levels found in most prostate tissues.

The high level of tissue specificity, plus the mRNA overexpression in 9 of 13 of the prostate tumor samples tested compared to the normal adjacent tissues are believed to make the PSG, prol01 (SEQ ID NO:1) a good diagnostic marker for detection of prostate cancer using mRNA.

- 18 -

What is Claimed is:

1. A method for diagnosing the presence of prostate cancer in a patient comprising:

- (a) measuring levels of PSG in a sample of cells,
5 tissue or bodily fluid obtained from the patient; and
- (b) comparing the measured levels of PSG with levels of PSG in a sample of cells, tissue or bodily fluid obtained from a control, wherein an increase in measured levels of PSG in the patient versus the PSG levels in the control is
10 associated with the presence of prostate cancer.

2. A method of diagnosing metastatic prostate cancer in a patient comprising:

- (a) measuring levels of PSG in a sample of cells,
15 tissue, or bodily fluid obtained from the patient; and
- (b) comparing the measured levels of PSG with levels of PSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in measured PSG levels in the patient versus the PSG levels in the control is associated
20 with a cancer which has metastasized.

3. A method of staging prostate cancer in a patient comprising:

- (a) identifying a patient suffering from prostate cancer;
- (b) measuring levels of PSG in a sample of cells,
25 tissue, or bodily fluid obtained from the patient; and
- (c) comparing the measured levels of PSG with levels of PSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in the measured levels of PSG versus the levels of PSG in the control is associated with
30 a cancer which is progressing and a decrease in the measured levels of PSG versus the levels of PSG in the control is associated with a cancer which is regressing or in remission.

- 19 -

4. A method of monitoring prostate cancer in a patient for the onset of metastasis comprising:

- (a) identifying a patient having prostate cancer that is not known to have metastasized;
- 5 (b) periodically measuring PSG levels in samples of cells, tissue, or bodily fluid obtained from the patient; and
- (c) comparing the periodically measured levels of PSG with levels of PSG in cells, tissue, or bodily fluid obtained from a control, wherein an increase in any one of the
- 10 periodically measured levels of PSG in the patient versus the levels of PSG in the control is associated with a cancer which has metastasized.

5. A method of monitoring changes in a stage of prostate cancer in a patient comprising:

- 15 (a) identifying a patient having prostate cancer;
- (b) periodically measuring levels of PSG in samples of cells, tissue, or bodily fluid obtained from the patient; and
- (c) comparing the measured levels of PSG with levels
- 20 of PSG in a sample of the same cells, tissue, or bodily fluid of a control, wherein an increase in any one of the periodically measured levels of PSG versus levels of PSG in the control is associated with a cancer which is progressing in stage and a decrease in any one of the periodically
- 25 measured levels of PSG versus the levels of PSG in the control is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4 or 5 wherein the PSG comprises SEQ ID NO:1.

SEQUENCE LISTING

<110> Ali, Shujath
 Salceda, Susana
 Sun, Yangming
 Cafferkey, Robert

<120> A Novel Method of Diagnosing, Monitoring and Staging
 Prostate Cancer

<130> DEX-0034

<140>

<141>

<150> 60/086,265

<151> 1998-05-21

<160> 7

<170> PatentIn Ver. 2.0

<210> 1

<211> 1936

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (1908)

<400> 1

```

aatggtatgc caacttaagt atttacaggg tggcccaaat agaacaagat gcactcgctg 60
tgattttaag acaagctgta taaacagaac tccactgcaa gagggngggc cgggccagga 120
gaatctccgc ttgtccaaga caggggccta aggagggtct ccacactgct gctaggggct 180
gttgcatttt ttattagta gaaagtggaa aggcctcttc tcaacttttt tcccttgggc 240
tgagaaattht agaatcagaa gtttcctgga gttttcaggc tatcatatat actgtatcct 300
gaaaggcaac ataattcttc cttccctcct tttaaaattt tgtgttcctt tttgcagcaa 360
ttactcacta aagggttca ttttagtcca gatttttagt ctggctgcac ctaacttatg 420
cctcgcttat ttagcccgag atctggtctt tttntgtnt tttttntnt tccgtctccc 480
caaagcttta tctgtcttga ctttttaaaa aagtttgggg gcagattctg aattgggcta 540
aaagacatgc atttttaaaa ctaggcaact tcttatttct ttcctttaaa aatacatagc 600
attaaatccc aaatcctatt taaagacctg acagcttgag aaggtcacta ctgcatttat 660
aggaccttct ggtggttctg ctgttacgtt tgaagtctga caatccttga gaatctttgc 720
atgcagagga ggtaagaggt attggatttt cacagaggaa gaacacagcg cagaatgaag 780
ggccaggctt actgaggctg tccagtggag ggctcatggg tgggacatgg aaaagaaggc 840
agcctaggcc ctggggagcc cagtccactg agcaagcaag ggactgagtg agccttttgc 900
aggaaaaggc taagaaaaag gaaaaccatt ctaaaacaca acaagaaact gtccaaatgc 960

```

```

tttgggaact gtgtttattg cctataatgg gtcccaaaaa tgggtaacct agacttcaga 1020
gagaatgagc agagagcaaa ggagaaatct ggctgtcctt ccattttcat tctgttatct 1080
caggtagagct ggtagagggg agacattaga aaaaaatgaa acaacaaaac aattactaat 1140
gaggtagcgt gaggcctggg agtctcttga ctccactact taattccgtt tagtgagaaa 1200
cctttcaatt ttcttttatt agaagggccg gcttactgtt ggtggcaaaa ttgccaacat 1260
aagttaatag aaagttaggc aatttcaccc cattttctgt ggtttgggct ccacattgca 1320
atgttcaatg ccacgtgctg ctgacaccga cgggagtact agccagcaca aaaggcaggg 1380
tagcctgaat tgctttctgc tctttacatt tcttttaaaa taagcattta gtgctcagtc 1440
cctactgagt actctttctc tccccctctc tgaatttaat tctttcaact tgcaatttgc 1500
aaggattaca catttcactg tgatgtatat tgtgttgtag ngaaaagaaa aaagtgtctt 1560
tgtttaaaat tacttggtt gtgaatccat cttgtctttt cccatttga actagtcatt 1620
aaccatctc tgaactggtg gaaaaacatc tgaagagcta gtctatcagc atctgacagg 1680
tgaattggat ggttctcaga accatttcac ccagacagcc tgtttctatc ctgtttaata 1740
aattagtttg ggttctctac atgcataaca aaccctgctc caatctgtca cataaaagtc 1800
tgtgacttga agtttagtca gcacccccac caaactttat ttttctatgt gttttttgca 1860
acatatgagt gttttgaaaa taaagtaccc atgtctttat taaaaanaa aaaaaagggc 1920
ggccgccgac tagtga 1936

```

<210> 2

<211> 637

<212> DNA

<213> Homo sapiens

<400> 2

```

gtaggggcag acttactgcc ttgaacgaaa gacgatggtc ctgctcagc ctactccaa 60
ttatgttcct ctaggtggg caggtagggg gtccagcttc ctgcttgctg gtggttcagg 120
tcatgctgcc agccttgctc ctctgacct gggccctacc cacggggaaa tgttccata 180
gcagaagaat cagccccaca gtgcaggggt gtgttagtgg ggaacgggct ctgggctcct 240
gtgggaacca gggaccccct atcttggtag cggctattgg atgtatcccc agtcatgcc 300
tgtgtctgtc ttggcccggt tggtcaccct gtgttcatct ctctcccagc catggcctct 360
caaactgggg ttttcgtctc cctatgaggg ggtcctggta tgtacgcgtt cgggtggccc 420
gcggtgcatg tctcccgtg cagtgcagtc tgggggtccc tggggccctg ggcccctcgt 480
aggatagaca gagcctgtcc taaccttccg gaagtgcag ctggggaggc cccttgctg 540
ctgaccttct gtgctcagga cgactaatcg gccacatgac caccactctg tcccatggga 600
ttcctagaga agtctcacta agagcccagc aactca 637

```

<210> 3

<211> 2693

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (2266)..(2512)

<220>

<221> unsure

<222> (586)

<220>

<221> unsure

<222> (1480)

<220>

<221> unsure

<222> (1532)

<220>

<221> unsure

<222> (1562) .. (1566)

<220>

<221> unsure

<222> (1569)

<220>

<221> unsure

<222> (1571)

<220>

<221> unsure

<222> (1631)

<400> 3

```

gctcctacag cgcctctgc gttacatag catccctatg gccactgtct cccttgatcc 60
ccacagccat cctaggagaa aggcagaatg tcataatttg ctaaaaggga tgctgaggct 120
ctgggaggga aagggacttg cctaaagccc cagggtgaag cagcatctct ggactcccag 180
tccagtgate ttgcccaata ctttgctgct tgcctatacc cctctaactt ggtcaacagc 240
acatcacagg gcaagcccaa tccctgcttc attttatat atgggcgctg gtccacagcc 300
ccactctcca gccatttgga aacaaaaaca gatgctattg ttcttcctta gagaacgtgg 360
ccagtggaga cggcacactg gaaatcagag tgaatgttct tgaaagaggg tcacgggtca 420
acaaggccca gccaaaggat gcagtagaac cattttcctt agaaatcttt gggagtgaag 480
taggcttcag ccactacca tccctgccct tgcggctacc actaccccat tagtttagac 540
agggctcggc ggggaggggt gtggagaaga aatgagcttg cctgtngccc ccaggctccc 600
tctgtcctag ctcaggtctg ggtgccattc tttaactctg tgtgtctcgt cagcacaca 660
tcacacacct tgctggtcac acagtacag actcgcctct gctcctgtgg tccagtggcc 720
ggacaccccc tgggatggct caaaggagtc aggacttgga agtggggaca tcagggtagc 780
tgaaggaaat ccacaccccc agagcatctc ggagttcaga ctctcagacc tgaagtaggc 840
gcccccgga ctgggctagg agttggacgg aatggaggat ggaggacagc gagaagaaag 900
gaagagaaat gcaaagtgtg ggcagccgcc aagagtgaat atagagggaa gtgtcatgca 960
agtgtctggc agaaggcggc aggtgggacg agccccacag cccctcctc aaaaacgacc 1020
acctccagga ctcagtgate cctggggggc aggtctctgc agccctcggc cacacgtggc 1080
tccggcacc atggtcccag tgcttggat ggagacggcc agttctggcg gccagatgtg 1140
gtgctctgga atccagtccc atttcttcc tggccacgcc tgttccagcg gcctctttgg 1200
ctgcattcag cccctactta cctggggacc ccggtctggg cacaagagca ccaggggggt 1260
agggcccaaa gggatcaggg gaagcctctg gcctggaggg tatggggcac gcttcccaaa 1320

```



```

gggcgggaccc ggcaggagga agcccaggag ctgggtcctg ccgcccagga gctggggccct 1380
gccaccacagg ccgggctagg gacatggcag ggcctgggca tcctgacgct ggacttgggc 1440
gacctgggag gcacaggag gggagagatg ggcggcccn acccagcgca gtgccggcca 1500
caccccaagg cggttgccag agcttaaggc cnggccccag caggagaaca tcccagctcc 1560
annnnnccnc nccgcagcca gtgtccttg tcaagctccc cccgtcactc cagggtgggag 1620
ccaccccggt naggggggtgt gccacttgcc ccagggcac tcctctgggc atcccggtg 1680
ggggattttg ggccgtggg gggcagtctc tggtagctgt gtgcgtcagg gatgctctgc 1740
acctgcaacc aggtgtcgtc cacgggcggg ggcattgggca tggtagacgt ggtcctgttg 1800
atgtcaccga tgatgctgag cgcctccttc agcgcgtggt gcatgtgcag catctcgtcg 1860
tgctgctgtg cctgctctgc caactcctcc atcagtgtgt tctggttccc acatgagtac 1920
atattggcca gcggctccga gatgatgaac tccggggtct gagagtgggc aaacagggaa 1980
gaaggtttgg acctggtgcc tgtgccgcc tggctgcctt gctggggccct tctgggactg 2040
tgcgctggac ttggagcccc ttggagtatg gcttttcaca cgggcttcta taccgcttcg 2100
actggaagat ccacctcccc actgcctttt ctactcaga tggggacacc gaggtccaga 2160
ggaaaagaca cctgtcaa atgtcacagatc tgggagggga cttaaagacct atcatgccaa 2220
gaggacacct gtctactcag tttttttttg gtggggcggg gggcgnnnnn nnnnnnnnnn 2280
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 2340
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 2400
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 2460
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnggagttg 2520
agttgatgcc tggtacacag agctctgtgg gtgggagtga gacaaaacac aggttcctga 2580
gctctgggga ccaagcaatg tcctctggtg aaaaaaatcc tggacttgct gccagaagat 2640
ttgcctctta cttgccatgt gctctgaata catttacctg ccctctggga aaa 2693

```

<210> 4

<211> 292

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (284)

<400> 4

```

aagaatatga gatttgctta gaaatgaagg actggaagga gcccacagag ttatttttta 60
aactatccag taaggcttag agggtttcaa tcagaaatat gtgttagggg aaaaaatgca 120
ctttttctat attaaaaaat attattttct tcttttaa atgtaaagcatt cctattgtga 180
agaattgaga aaatacagaa aagtacaaag aaaaacatta cctacaactc caccatccgt 240
gattatcact gttcacattt gtggctcatt tttcagtatk tctnttattt aa 292

```

<210> 5

<211> 2694

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (52)

<220>
<221> unsure
<222> (74)

<220>
<221> unsure
<222> (76)

<220>
<221> unsure
<222> (80)

<220>
<221> unsure
<222> (92)

<220>
<221> unsure
<222> (97)

<220>
<221> unsure
<222> (123)

<220>
<221> unsure
<222> (132)

<220>
<221> unsure
<222> (173)

<220>
<221> unsure
<222> (217)

<220>
<221> unsure
<222> (257)

<220>
<221> unsure
<222> (2539)

<400> 5
tactatattg ctcagcattt ctaagtattc tctaagtgc ctttatttat gntttaaaat 60
agctctctta ccngntgcg ncgactagaa gancttgntt taggaaacaa tgaaatatat 120

```

aanttgccag antcaattgg agccctctta catctaaaag atctctgggt ggntggaaat 180
caactgtcag aattacctca ggaaatagga aatctgnaga acctgctgtg tttagatgtc 240
tctgaaaaca ggttggnaaag acttcctgaa gaaatcagt gcctgacttc attaacggat 300
ttagtcatctt ccagaaactt attagaaacg attccggatg gcattggaaa actaaagaaa 360
ctgtcaatct tgaaggtgga tcagaataga ctcacacagt tgcctgaagc agttggggaa 420
tgtgaaagtc tactgagtt agttcttaca gaaaatcagc tcctgacct gcctaaaagc 480
attggaanaac taaagaagtt gagcaacttg aatgcagaca gaaataaatt agtgcctta 540
ccaaaagaga tcggcggtg ctgcagctc actgtgttct gtgtacgtga caacagacta 600
actcggtac ctgcagaggt gtcacaggca acagaacttc atgtcctgga tgtggcagg 660
aacaggttgc tgcactacc tttatccctg actgcctga agttgaaggc tctgtggcta 720
tctgacaacc agtccagcc cctgcttaca ttccagacag acacagacta caccacagga 780
gagaagattt taactgtgt cttacttct cagctgcct ctgaacctac ttgtcaagag 840
aatctgcctc gctgtggtgc actggagaac ttggtaaatg atgtctctga tgaagcctgg 900
aacgagcgtg ctgtcaacag agtcagtgcg atccgatttg tggaggatga gaaagatgaa 960
gaagacaatg agacgagaac acttctaagg cgagccactc cacaccagg ggagttaaag 1020
cacatgaaaa agacagtggg gaatttacgg aatgacatga atgtctgtaa aggactggac 1080
tcaaacaaaa acgaggtcaa tcatgccatt gaccgagtga ccacttctgt gtagagtttc 1140
acctccaagt ttactctct gtgtcttct ctgctgtcga gacgttctg tctgttccc 1200
gggagcctca cgtgctcctt gtcctaacca gccccgcgc gccatcttc cgtggagtgt 1260
ggggaagctg ctgtctccca ggaagtgcct tactcatccc gcaaccagtc agcgaccag 1320
tggtctcccg gtgtgatttt ttttttttt aatttcagtt gtttgtaata agtagaatac 1380
actactgtaa acatacgacc tttgttttg tcttatgttg gggtaaagga aagcaggaag 1440
gggaattttt atcctctcc cttccgtaaa gtgctgggat attttgaatc cccaagtct 1500
ccttggacct actgatgaga gatagtttta tgtatgggga aaaatggata ctttttaaac 1560
cttttttggc agctcagatg gtgtaaattt taaaattttg tataggtatt tcataacaaa 1620
aatatgtatt tctttttgt tattttatct tgaanaacgg acatatttta gtatttgtgc 1680
agaaaaacaa gtcctaaagt atttgtttt atttgtacca tccacttgtg cttactgtg 1740
tcctgtgtca tgtccaatca gttgtaaaca atggcatct tgaacagtgt gatgagaata 1800
ggaatgtggt gttttaaagc agtgttgcat tttaatcagt aatctacctg gtggatttgt 1860
ttttaacca aaagatgaat tatcaatgat ttgtaattat atcggttgat ttttttgaa 1920
aagatgaacc aaaggatttg actgctaata tttatttct tacacttttt ttctgaataa 1980
gtctctcata atgagtgcag tgtcagactg tgcctactct gatggtatgt gccatttgta 2040
aaataaaata gagcagaaaa acacaaaaag agaactctgg ttcagacatt cagtgggcaa 2100
gtaaattatg gactgcaaaa taatgatttt tattcaagaa agctttaaaa gttttatata 2160
cagatatata accacaataa agcaaaaata cctactatca aaatagaaat gttgctatct 2220
ttataagtgc aatttaattt gtaaatagag tttgaatcaa agtatcacia aatactgctt 2280
caagatttaa ttttaaatct gctaatttaa gggatatttg gaaaagtttt ggtgtgtttc 2340
tgttgatttc tttttgtat gctgtgataa aagagaaatg aaaagtgcc gtcactgtgt 2400
ggtgtctagg aaaatcatat atatttttt ctccaagaaa taaattcatc ctggacattg 2460
gccatacagc tttttaaaat tattactttg tatgttcaag tgatagcagg tagccaaatt 2520
ctttgacagt gtgctctgnt ctgttaaata tctaaattac ccgtcagttg tgagtacact 2580
cctgtgggac ttgcattcac atggggcaga gcccagaatt gcctttgact ctggctagta 2640
attttgggtt gtggctatct ggccaattgg actccttata aaccgtctt caac 2694

```

<210> 6

<211> 1335

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (17)

<400> 6

```

tcatatagta ggaaganaag cacctagggt tgaggccagg gctggctgct gtcagaacct 60
agggcctccc ctgccttgct ccacacctgg tcaggggaga gaggggagga aagccaaggg 120
aagggaacct actgaaaaca aacaagctgg gagaagcagg aatctgcgct cgggttccgc 180
agatgcagag gttgaggtgg ctgcgggact ggaagtcac gggcagaggt ctcacagcag 240
ccaaggaacc tggggcccg cctcccccc tccaggccat gaggattctg cagttaatcc 300
tgcttgctct ggcaacaggg cttgtagggg gagagaccag gatcatcaag gggttcgagt 360
gcaagcctca ctccagccc tggcaggcag ccctgttcga gaagacgagg ctactctgtg 420
gggcgacgct catcgcccc agatgggtcc tgacagcagc cactgcctc aagccgtggc 480
cgctacatag ttcacctggg gcagcacaac ctccagaagg aggagggctg tgagcagacc 540
cggacagcca ctgagtcctt cccccacccc ggcttcaaca acagcctccc caacaaagac 600
caccgcaatg acatcatgct ggtgaagatg gcatcgccag tctccatcac ctgggctgtg 660
cgacccctca ccctctctc acgctgtgtc actgctggca ccagctgcct catttccggc 720
tggggcagca cgtccagccc ccagttacgc ctgcctcaca ccttgcatg cgccaacatc 780
accatcattg agcaccagaa gtgtgagaac gcctaccccg gcaacatcac agacaccatg 840
gtgtgtgcca gcgtgcagga agggggcaag gactcctgcc agggtgactc cggggggcct 900
ctggtctgta accagtctct tcaaggcatt atctcctggg gccaggatcc gtgtgcgac 960
acccgaaagc ctggtgtcta cacgaaagtc tgcaaatatg tggactggat ccaggagacg 1020
atgaagaaca attagactgg acccaccac cacagcccat caccctccat ttccacttgg 1080
tgtttggttc ctgttcactc tgtaataaag aaaccctaag ccaagaccct ctacgaacat 1140
tctttgggccc tctggacta caggagatgc tgtcacttaa taatcaacct ggggttcgaa 1200
atcagtgaga cctggattca aattctgcct tgaaatattg tgactctggg aatgacaaca 1260
cctggtttgt tctctgttgt atccccagcc ccaaagacag ctctgccat atatcaagtt 1320
tcaataaata tttct 1335

```

<210> 7

<211> 1079

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (268)

<220>

<221> unsure

<222> (688)

<220>

<221> unsure

<222> (700)

<400> 7

tttttgaaga atgccctgca aggcatacaac tggaatgtgt ttattaccaa acaagacaga 60
agagaaccag ggcctgactt ggcagtggcc ccaggctgca tgggctcagg taggctcaga 120
ccggccccag gagtgggaga gccagagaa gagggaaaaa gagtagtggc caggaggggt 180
ctggctggga catgccactc tgggccatca gcttctggat cactcaaag tggtggtga 240
tattggtgta gacaccgggc cgattggncc gaccacagcc cactccccag ctcacgactc 300
caatctgata ccacagtcca ttcttggttac aggccaaagg tccacctgag tcaccgaagc 360
aggcatcctt cccgccttgg gcattgccag cacaaccat gtctcaaag atgtccttgc 420
ggaaactgta cttgaggaag aggtggttgc acatagagtt gtttatgatg gcgacctgaa 480
cttcctggag ggtgtgggga gatggcagtg cctcatcctc tttgatgtac cccagccag 540
tcaccagca gtctgtccgg ttctcaaact caaatgtgga ggcctggaga cagatgggt 600
ggatgtgttt agttaggtg acaggtgcag acagcttcac caaggcaatg tcatagggtg 660
aattccccag ttagcgaggc ctcagatnga tattegatan gaagtaacgg gtgtagtagg 720
cctgcaggct ccagaaggat ggcattggaag tcagctggcc aaactggacc atccaccgg 780
agggatcact aaggtcacta taggtttcaa agcagtgcgc cgccgtgagt gccagcgg 840
ggctgagcag gctcactccg catacgtggg aatcccacag gcgcaggctc ccctgccacg 900
gccaacgccc gagttcggcg tcctctccac ccacgatgcg cgacgtgatg acccgctggc 960
cgcatggtcc tgataagggc gccgcctcct gcgactccgg cttcctgagt ccagcccag 1020
ccagcagcag cgccagcagc agcgccccgc gcgcgcccac ggcctcctct cccgcgggtg 1079

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10548

| A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : C12Q 1/68; G01N 33/574 US CL : 435/6, 7.23 According to International Patent Classification (IPC) or to both national classification and IPC | | | | | | | | | | | | | | |
|--|--|--|--|---|--|--|--|--|---|---|--|--|--|--|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/6, 7.23 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DIALOG, APS | | | | | | | | | | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | | | | | | | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | | | | | | | | | | | |
| X | US 5,506,106 A (CROCE et al.) 09 April 1996, col. 1, lines 50-65. | 1-5 | | | | | | | | | | | | |
| X | DEGUCHI, T. et al. Detection of Micrometastatic Prostate Cancer Cells in the Bone Marrow of Patients with Prostate Cancer. British Journal of Cancer. 1997, Vol. 75, No. 5, pages 634-638, especially page 634. | 1-5 | | | | | | | | | | | | |
| P,Y | AN, G. et al. Cloning of Prostate-Specific Genes that are Suppressed in Metastatic Prostate Cancer by a PCR Southern Differential Hybridization Method. Cell and Tumor Biology. March 1998, Vol. 39, page 208, especially page 208. | 1-6 | | | | | | | | | | | | |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | | | | | | | | | | | | | |
| <table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A* document defining the general state of the art which is not considered to be of particular relevance</td> <td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*E* earlier document published on or after the international filing date</td> <td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>*G* document member of the same patent family</td> </tr> <tr> <td>*O* document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>*P* document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table> | | | * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | *E* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *G* document member of the same patent family | *O* document referring to an oral disclosure, use, exhibition or other means | | *P* document published prior to the international filing date but later than the priority date claimed | |
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | | | | | | | | | | | | | |
| *E* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | | | | | | | | | | | | | |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *G* document member of the same patent family | | | | | | | | | | | | | |
| *O* document referring to an oral disclosure, use, exhibition or other means | | | | | | | | | | | | | | |
| *P* document published prior to the international filing date but later than the priority date claimed | | | | | | | | | | | | | | |
| Date of the actual completion of the international search 12 AUGUST 1999 | | Date of mailing of the international search report 09 SEP 1999 | | | | | | | | | | | | |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230 | | Authorized officer <i>D. Lawrence Fer</i> YVONNE EYLER Telephone No. (703) 308-0196 | | | | | | | | | | | | |